

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) A method of producing a genetically modified mammalian cell, said method comprising the steps of:

(a) inserting into one or more mammalian cells an artificial chromosome comprising a cassette which includes a first region of homology having at least 90% sequence identity to a first region of an endogenous chromosome of said one or more mammalian cells, a selectable marker, and a second region of homology having at least 90% sequence identity to a second region of said endogenous chromosome under conditions that result in homologous recombination between said artificial chromosome and said endogenous chromosome and integration of said cassette into said endogenous chromosome of one or more mammalian cells; and

(b) selecting a mammalian cell in which said homologous recombination occurs, thereby selecting a genetically modified mammalian cell.

2. (Currently amended) ~~The method of claim 1, wherein prior to step (a) said method further comprises the step of:~~ A method of producing a genetically modified mammalian cell, said method comprising the steps of:

(a) culturing a host cell that has (i) a linear DNA molecule comprising said cassette a cassette which includes a first region of homology having at least 90% sequence identity to a first region of an endogenous chromosome of said mammalian cell, a selectable marker, and a second region of homology having at least 90% sequence identity to a second region of said endogenous chromosome and (ii) an artificial chromosome comprising a first nucleic acid sequence that is has at least 90% identical sequence identity to said first and second regions region of homology and a second

nucleic acid sequence that has at least 90% sequence identity to said second region of homology under conditions that result in homologous recombination between said linear DNA molecule and said artificial chromosome, thereby generating an artificial chromosome comprising said cassette;

(b) inserting into one or more mammalian cells said artificial chromosome comprising said cassette under conditions that result in homologous recombination between said artificial chromosome and said endogenous chromosome and integration of said cassette into said endogenous chromosome of said one or more mammalian cells; and

(c) selecting a mammalian cell in which said homologous recombination occurs, thereby selecting a genetically modified mammalian cell.

3. (Original) The method of claim 2, wherein said linear DNA molecule is introduced into said host cell by transformation.

4. (Currently amended) The method of claim 2, wherein prior to step (a) said linear DNA molecule is generated in said host cell by insertion of a circular vector comprising the sequence of said linear DNA molecule is inserted into said host cell and cleavage of said vector is cleaved to generate said linear DNA molecule inside said host cell.

5. (Original) The method of claim 1, wherein said first and second regions of said endogenous chromosome are contiguous.

6. (Original) The method of claim 5, wherein said first and second regions of said endogenous chromosome are part of the same exon or the same promoter.

7. (Original) The method of claim 1, wherein said first and second regions of said

endogenous chromosome are not contiguous.

8. (Original) The method of claim 7, wherein said first and second regions of said endogenous chromosome are part of different exons.

9. (Currently amended) The method of claim 1, wherein the integration of said cassette into said endogenous chromosome of said one or more mammalian cells reduces the activity of ~~the~~ a protein encoded by a nucleic acid of interest that includes said first or said second region of said endogenous chromosome.

10. (Currently amended) The method of claim 9, wherein ~~the amount of~~ said activity of said functional protein encoded by said nucleic acid of interest decreases by at least 25%.

11. (Previously presented) The method of claim 1, wherein said selectable marker is a reporter gene, and wherein said cassette is integrated into said endogenous chromosome of said one or more mammalian cells such that said reporter gene after integration is operably linked to an endogenous promoter of interest of said one or more mammalian cells, thereby generating a genetically modified mammalian cell that expresses said reporter gene under the control of said promoter.

12. (Previously presented) The method of claim 1, wherein said selectable marker is a nucleic acid encoding a detectable protein, and wherein said cassette is integrated into said endogenous chromosome of said one or more mammalian cells such that said nucleic acid after integration is operably linked to an endogenous nucleic acid of said one or more mammalian cells encoding a protein of interest, thereby generating a genetically modified mammalian cell that expresses a fusion protein comprising said detectable protein and

protein of interest or fragment thereof.

13. – 17. (Canceled)

18. (Original) The method of claim 1, wherein said mammalian cell is an embryonic stem cell.

19. (Original) The method of claim 1, wherein said mammalian cell is a somatic cell.

20. – 39. (Canceled)